Response of Lewis Flax Seedlings to Inoculation With Arbuscular Mycorrhizal Fungi and Cyanobacteria

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Abstract: Forbs comprise an important though understudied component of western rangelands. Little is known about the dependence of forb species on associated soil microorganisms such as arbuscular mycorrhizal fungi. In 1996 and 1998, we conducted two experiments examining the response of Lewis flax (*Linum lewisii* Pursh) to inoculation with arbuscular mycorrhizal fungi under a variety of soil conditions. Lewis flax was found to be highly dependent on mycorrhizae when grown in sandy soils, regardless of soil nutrient levels. Mycorrhizal plants had 10 times the root biomass and 16 times the shoot biomass of nonmycorrhizal controls. The addition of mycorrhizae also significantly decreased root:shoot ratios and significantly increased plant tissue concentrations of P, K, and Zn. The addition of cyanobacterial inoculum to the soil increased plant survival at low soil fertility, but had no effect on seedling growth. In the second experiment, three accessions of flax were grown in sand, peat, and a sand and peat mixture fertilized with high levels of a slow-release fertilizer. All three flax accessions responded positively to mycorrhizae in the sand and mixed soils, growing five and three times larger, respectively. In contrast, no significant response was observed in the peat soil. These findings indicate that mycorrhizal dependence is complex, and can be influenced by a number of factors, including soil nutrient status and organic matter content.

Introduction

Native forb species are prized both for their colorful contribution to western landscapes and for their importance in the diets of many herbivorous wildlife species. While early revegetation efforts focused primarily on the use of grass and shrub species, the use of herbaceous flowering plant seed in revegetation mixes has become increasingly common. As a result, the establishment requirements of forbs are becoming a research priority (Kitchen 1994).

North American Lewis flax (*Linum lewisii*) has wide distribution over the western half of the United States, Canada, and Northern Mexico (Mosquin 1971). Lewis flax and its close relative, *L. perenne*, have been used in revegetation and restoration plantings and show horticultural potential in xeriscaped gardens (Kitchen 1995; McArthur 1988). Other flax species are known to benefit from mutualistic associations with arbuscular mycorrhizal fungi (Thingstrup and others 1998). The purpose of this research was to examine the relative response of Lewis flax seedlings to inoculation with arbuscular mycorrhizal fungi (AMF) under a variety of soil conditions, including inoculation with biological soil crust organisms.

Methods

Experiment 1

Olympian 300XL pots (Hummert Int., Earth City, MO) were filled with a low-fertility bank sand that had been amended to one of three fertilizer levels. The sand was steam sterilized at 65 °C for 1 hour to kill any indigenous mycorrhizal fungi that might be present, and 2.4 g ammonium nitrate per 30 gallons of sand was added to raise the nitrogen level to 10 ppm. Medium and high fertilizer levels were amended with Osmocote 17-7-12 (The Scotts Company, Marysville, OH), formulated for 12 to 14 months continuous fertilization, applied at a rate of 5 and 9 ounces per cubic foot, respectively. Each pot received one of four inoculation treatments: algal inoculation, mycorrhizal inoculation, dual inoculation of both algae and mycorrhizae, and a noninoculated control. Mycorrhizal inoculation consisted of 1 teaspoon (approximately 100 spores) of pot-cultured mycorrhizal inoculum applied 1 to 2 inches below the soil surface. The inoculum was produced from soil collected from

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a mixed desert shrub community near Toquerville, UT, that had been planted with ornamental corn seed (Carpenter Seed Co., lot 125). Nonmycorrhizal pots received an equal amount of inoculum that had been sterilized by autoclaving. Soil crust inoculum consisted of pelletized algal inoculum of the genera *Microcoleus* and *Nostoc* (Buttars and others 1998) ground to the consistency of cornmeal using a wheat grinder and applied at a rate of 1.6 g per pot, sprinkled by hand over the top of the soil at the time of planting.

Pots were sown with flax seed collected near Potosi Pass, west of Las Vegas, NV. Flax plants were thinned to one plant per pot upon emergence and grown for 15 weeks in a thoroughly cleaned, temperature-controlled greenhouse. At harvest, shoots were excised, dried, weighed, and ground for analysis of mineral content. Tissue samples were analyzed at the Soil and Plant Analysis Laboratory, Brigham Young University, Provo, UT, using atomic absorption and micro-Kjeldahl procedures (Johnson and Ulrich 1959). Results are only reported for plants grown at high soil fertility, as nonmycorrhizal plants at medium and low fertility produced insufficient biomass for mineral analysis. Roots were washed free of sand, dried at 60 °C, weighed, and stored at room temperature pending examination of mycorrhizal colonization (Tarkalson and others 1998).

Data were analyzed using the General Linear Models procedure on SAS, version 6.11 (SAS Institute 1993). Soil fertility, algal treatment, and AMF treatment were used as main effects in the model. Because of significant interaction terms involving soil fertility, the effects of algal and AMF inoculation treatments were also analyzed separately by soil fertility level. Survival data were analyzed using a 3 x 2 x 2 contingency table, later subdivided by soil fertility level.

Experiment 2

Seeds of three accessions of native Lewis flax (Potosi Pass, NV; Provo, UT; and Asotin, WA) were sown into SC-10 Super Cells (Stuewe & Sons, Corvallis, OR) containing one of three soil mixtures: (1) low-fertility bank sand that had been steamed for 2 hours at 77 °C, (2) a peat-based commercial potting soil, and (3) a mixture composed of half sand and half commercial potting soil. Mycorrhizal inoculum of the species *Glomus intraradices*, obtained from Tree of Life Nursery, Capistrano, CA, was added to one-half of the cells approximately 2 inches below the soil line, at a rate of 1 tablespoon per cell.

Fourteen replicate cells were used for each of the 18 soil/accession/inoculum combinations. Plants were thinned to one plant per cell and grown for 18 weeks in a temperature-controlled greenhouse. Each cell (plant) was fertilized using 1 tablespoon of Osmocote 17-7-12 (The Scotts Company, Marysville, OH). Plant height was recorded at the end of 9, 14, and 18 weeks growth. After 18 weeks, shoots were excised, dried, and weighed. Roots were washed free of soil, dried at 60 °C, weighed, and stored at room temperature for later examination of mycorrhizal colonization.

Data were analyzed using the General Linear Models procedure on SAS, version 6.11 (SAS Institute 1993). Soil type, plant accession, and AMF treatment were used as main effects in the model. Data were also analyzed by soil type and accession.

Results

Experiment 1

Survival of experimental plants was influenced by soil fertility level and algal inoculation (X^2 = 47.8; P < 0.001). Overall, survival was greatest at low fertility, followed by medium fertility (fig. 1). Inoculation with algae significantly improved survival at low fertility (X^2 = 12.6; P < 0.001), but significantly decreased survival at high fertility (X^2 = 11.1; P < 0.001).

Growth of flax plants was significantly affected by both soil fertility and inoculation with mycorrhizal fungi (table 1).

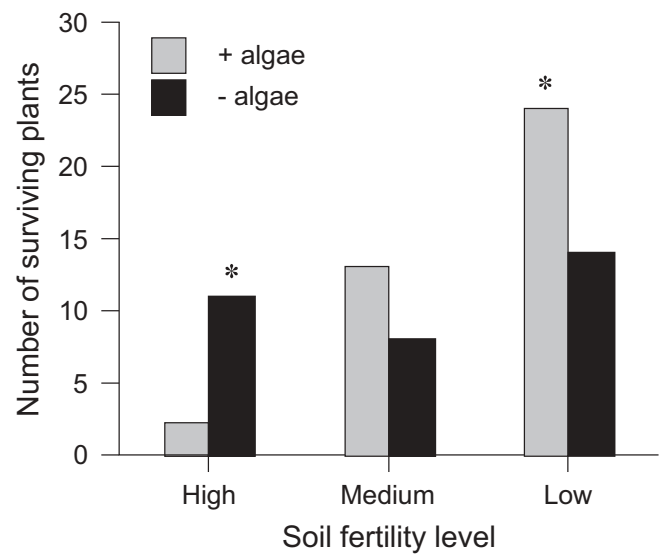

Figure 1—Effect of algal treatment on survival of Lewis flax plants (experiment 1). Bars marked with an asterisk indicate significant differences (P ≤ 0.05) in plant survival.

Table 1—Attained significance values for treatment effects on Lewis flax growth measures by fertilizer level. Data are from experiment 1.

Fertility/source	Shoot biomass	Root biomass	r:s ratio
High fertility			
AMF inoculation	0.0135[a]	0.0895	0.0025[b]
Algal inoculation	NE[c]	NE[c]	NE[c]
AMF x algae	NE[c]	NE[c]	NE[c]
Medium fertility			
AMF inoculation	.0028[b]	.0043[b]	.0003[b]
Algal inoculation	.7954	.8350	.1068
AMF x algae	.7169	.8195	.3659
Low fertility			
AMF inoculation	.0001[b]	.0001[b]	.1395
Algal inoculation	.0588	.1693	.0226[a]
AMF x algae	.1386	.2598	.9970

[a] Significant at P ≤ 0.05.
[b] Significant at P ≤ 0.01.
[c] NE = nonestimable due to missing data.

Shoot biomass was significantly greater for mycorrhizal plants at all soil fertility levels (fig. 2). Root biomass was also significantly greater at medium and low soil fertility. Root: shoot ratios increased with decreasing soil fertility, but were consistently lower for mycorrhizal plants. Mycorrhizal plants also had higher tissue concentrations of P, K, Zn, and Mn (table 2). Calculated values for mycorrhizal dependence of flax (Plenchette and others 1983) ranged from 72 to 98 percent.

Inoculation with blue-green algae increased root:shoot ratios at low and medium fertility levels (table 1; fig. 2), suggestive of some competition between plants and microorganisms. Algal inoculation also significantly increased plant tissue concentrations of K (2.06 versus 1.45 percent; $F = 9.2$; $P = 0.01$).

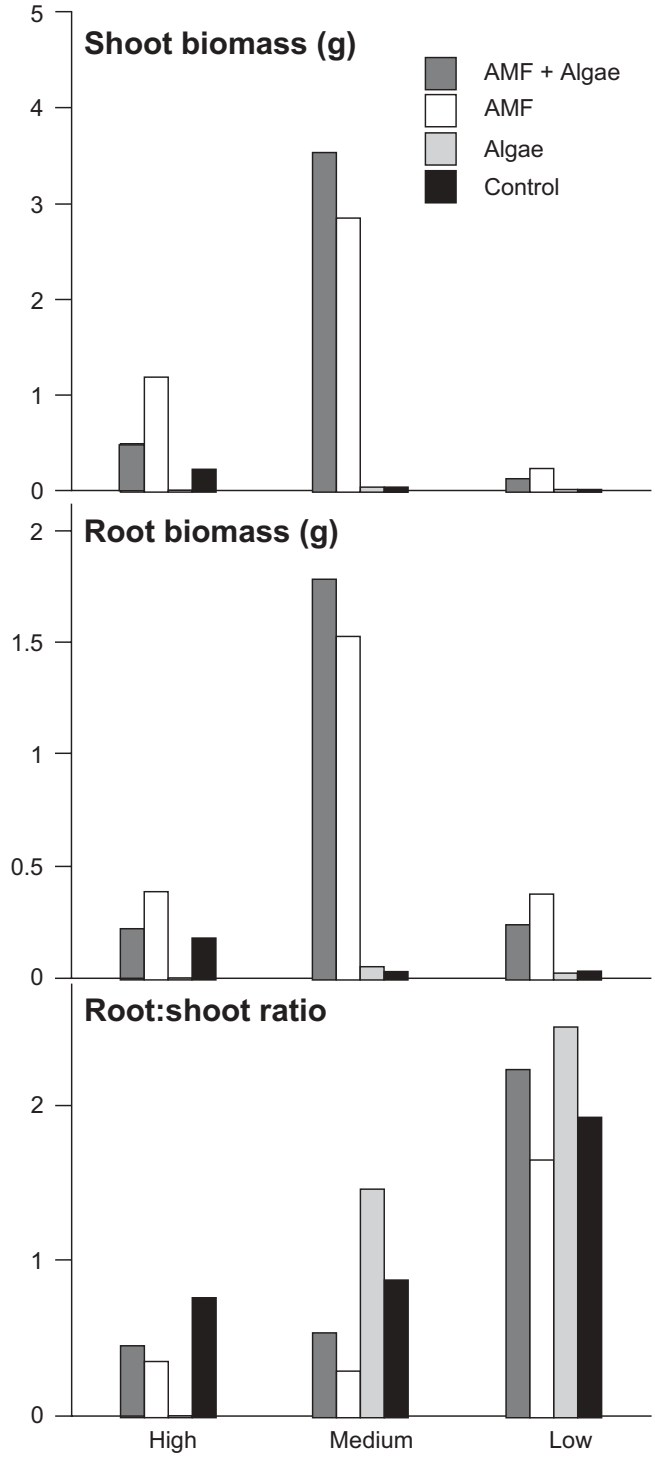

Figure 2—Effect of inoculation treatments on growth of Lewis flax plants (experiment 1).

Experiment 2

While flax accessions differed in inherent growth rate, all accessions grew better with the addition of mycorrhizal inoculum when grown in either the sand or sand and peat mixture (fig. 3). Mycorrhizal plants were significantly taller and had greater shoot and root biomass (table 3), as well as total biomass, in these two soils. Mycorrhizal dependency values for these soils ranged from 78 to 96 percent, indicating that Lewis flax is strongly dependent on the fungus. In contrast, no significant differences in growth occurred in the peat-based potting soil. Dependency values in the peat soil ranged from 1 to 7 percent, indicating little or no dependence under these growing conditions.

Conclusions

1. Lewis flax may be characterized as mycorrhizal dependent. In inorganic media, growth of flax was significantly enhanced by inoculation, having dependency values ranging from 78 to 98 percent. The congener, *L. usitatissimum*, is also mycorrhizal dependent, exhibiting increased growth, reproduction, and resistance to pathogens in the presence of the fungus (Dugassa and others 1996; Thingstrup and others 1998).

2. In contrast, dependency values in organic soil ranged from 1 to 7 percent. The addition of organic matter through the peat-based potting mixture in many ways mimicked the response to P fertilization reported in previous studies. Mycorrhizal dependency may be influenced by a number of soil factors, including nutrient level, soil microorganisms, and organic matter content (Hetrick and others 1986; Schweiger and others 1995).

3. Inoculation with crust-forming algae increased survival at low soil fertility, but also increased root:shoot ratios. A shift in biomass allocation toward increased root mass suggests an increase in belowground competition for soil resources. Some competition between plant roots and soil microorganisms is likely, particularly during crust establishment.

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Table 2—Elemental tissue concentrations for mycorrhizal and nonmycorrhizal Lewis flax growing at high soil fertility. Nonmycorrhizal plants growing at medium and low fertility yielded insufficient biomass for elemental analysis. Attained significance values (P) from statistical analyses are given below. Significant differences are in boldface. Data are from experiment 1.

	n	N	P	K	Ca	Mg	Zn	Fe	Mn	Cu	Na
		percent					*ppm*				
+ AMF	4	4.16	0.33	2.01	2.73	0.69	17.47	203.1	67.3	10.83	743.5
– AMF	2	3.85	.11	.91	3.07	.78	4.43	280.3	128.5	10.47	740.3
P value		.3851	**.0147**	**.0389**	.5799	.4601	**.0310**	.2994	**.0082**	.8881	.9922

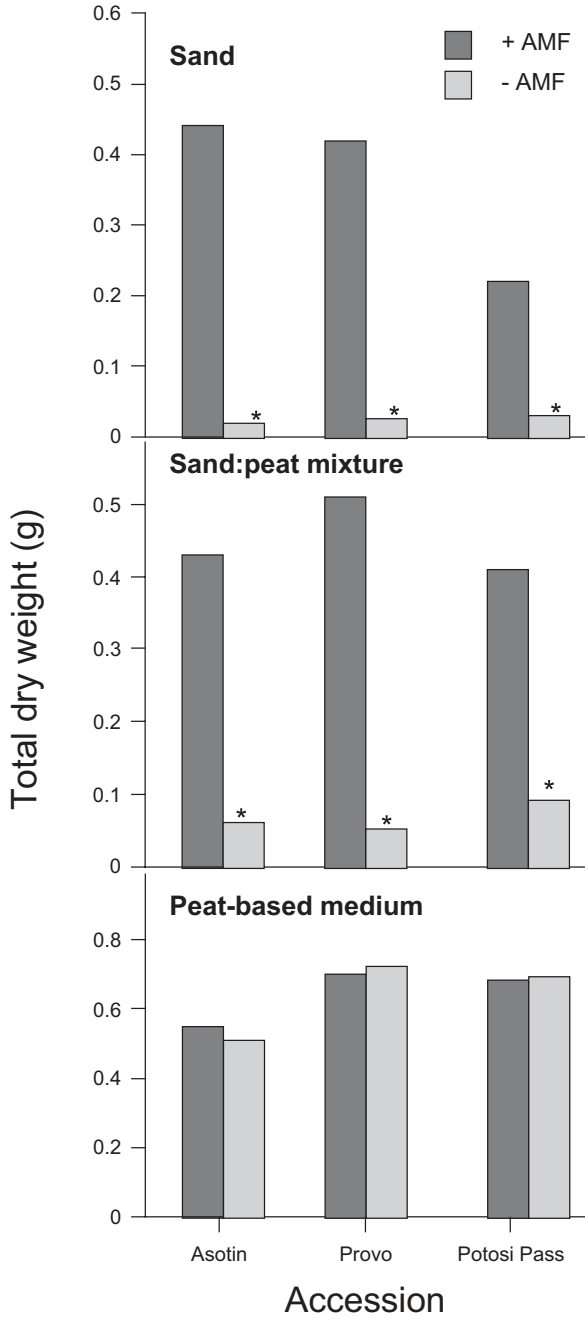

Figure 3—Effect of mycorrhizal fungi on growth of Lewis flax in three contrasting growth media (experiment 2). Bars marked with an asterisk indicate significant differences (P ≤ 0.05) between mycorrhizal and nonmycorrhizal plants.

Table 3—Attained significance values from ANOVAs on growth measures of three accessions of mycorrhizal and nonmycorrhizal flax plants grown in sand, a peat-based potting soil, and a 1:1 mixture of the two. Data are from experiment 2.

Soil/accession	Height	Shoot biomass	Root biomass	Total biomass	Root/shoot ratio
	cm	----------	*g*	----------	
Sand					
Asotin	0.0001	0.0035	NS	0.0300	NS
Provo	.0001	.0051	.0043	.0038	NS
Potosi Pass	.0001	.0180	.0076	.0078	NS
Sand:peat					
Asotin	.0001	.0001	.0004	.0001	.0005
Provo	.0001	.0001	.0001	.0001	.0009
Potosi Pass	.0006	.0046	.0111	.0055	.0001
Peat					
Asotin	NS[a]	NS	NS	NS	NS
Provo	NS	NS	NS	NS	NS
Potosi Pass	NS	NS	NS	NS	NS

[a] Not significant at the 0.05 level.

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